EPILEPSY CURRENTS

Current Literature

In Basic Science

KCNQ2 Potassium Channel Epileptic Encephalopathy Syndrome: Divorce of an Electro-Mechanical Couple?

***KCNQ2* Encephalopathy: Emerging Phenotype of a Neonatal Epileptic Encephalopathy.**

Weckhuysen S, Mandelstam S, Suls A, Audenaert D, Deconinck T, Claes LR, Deprez L, Smets K, Hristova D, Yordanova I, Jordanova A, Ceulemans B, Jansen A, Hasaerts D, Roelens F, Lagae L, Yendle S, Stanley T, Heron SE, Mulley JC, Berkovic SF, Scheffer IE, de Jonghe P. *Ann Neurol* 2012;71:15–25.

OBJECTIVE: *KCNQ2* and *KCNQ3* mutations are known to be responsible for benign familial neonatal seizures (BFNS). A few reports on patients with a KCNQ2 mutation with a more severe outcome exist, but a definite relationship has not been established. In this study we investigated whether *KCNQ2/3* mutations are a frequent cause of epileptic encephalopathies with an early onset and whether a recognizable phenotype exists. METHODS: We analyzed 80 patients with unexplained neonatal or early-infantile seizures and associated psychomotor retardation for *KCNQ2* and *KCNQ3* mutations. Clinical and imaging data were reviewed in detail. RESULTS: We found 7 different heterozygous *KCNQ2* mutations in 8 patients (8/80; 10%); 6 mutations arose de novo. One parent with a milder phenotype was mosaic for the mutation. No *KCNQ3* mutations were found. The 8 patients had onset of intractable seizures in the first week of life with a prominent tonic component. Seizures generally resolved by age 3 years but the children had profound or less frequently severe, intellectual disability with motor impairment. Electroencephalography (EEG) at onset showed a burst-suppression pattern or multifocal epileptiform activity. Early magnetic resonance imaging (MRI) of the brain showed characteristic hyperintensities in the basal ganglia and thalamus that later resolved. INTERPRETATION: *KCNQ2* mutations are found in a substantial proportion of patients with a neonatal epileptic encephalopathy with a potentially recognizable electroclinical and radiological phenotype. This suggests that *KCNQ2* screening should be included in the diagnostic workup of refractory neonatal seizures of unknown origin.

Commentary

The potassium channels expressed from the *KCNQ* genes are standouts for epileptologists, in that they are both mutated in human epilepsy and principal targets of an approved antiepileptic drug (ezogabine/retigabine) (1, 2). Mutations in two closely related subunits, KCNQ2 or KCNQ3, cause benign familial neonatal seizures (BFNS), an autosomal dominant syndrome characterized by seizures in the first weeks or months of life that remit and are followed by normal motor and cognitive development. Weckhuysen et al. now extend the *KCNQ2* phenotypic spectrum, describing a set of seven novel mutations in eight patients with persistent epilepsy and psychomotor disability.

The initial clinical presentation of KCNQ2 encephalopathy is instantly recognizable as akin to two severe neonatal epileptic encephalopathies, Early Myoclonic Encephalopathy (EME) and Ohtahara syndrome, which are characterized by tonic and myoclonic seizures with EEG burst suppression and onset within the first week of life (3). Patients with EME and Ohtahara syndrome typically undergo exhaustive evaluation for metabolic or structural etiologies. Treatment-resistant seizures usually continue throughout an evolution to other severe age-dependent encephalopathies such as West and Lennox-Gastaut syndromes. In contrast, the patients of Weckhuysen et al. largely became seizure-free (under anticonvulsant treatment), after approximately 1 to 3 years but exhibited persistent cognitive and motor impairment. The EEG evolved to multifocal epileptiform discharges within weeks to months and was normal in four patients at the last follow-up test. The evidence that the new mutations are pathogenic in this novel syndrome is strong but would be enhanced through description of more patients and functional studies of the mutations.

Even without such additional work, however, earlier studies suggest testable, potentially complementary, explanations why these mutations might cause severe phenotypes. Each of these hypotheses invokes a pathogenic "divorce" of components that are tightly coupled in the healthy state. The first such obligatory linkages are found within each KCNQ2 subunit protein. All voltage-gated channels are equipped to (1) sense the membrane potential, (2) open and close their pore in response to membrane voltage changes, and (3) selectively allow ions to pass. The connection between membrane voltage-sensing and the pore's gates is termed

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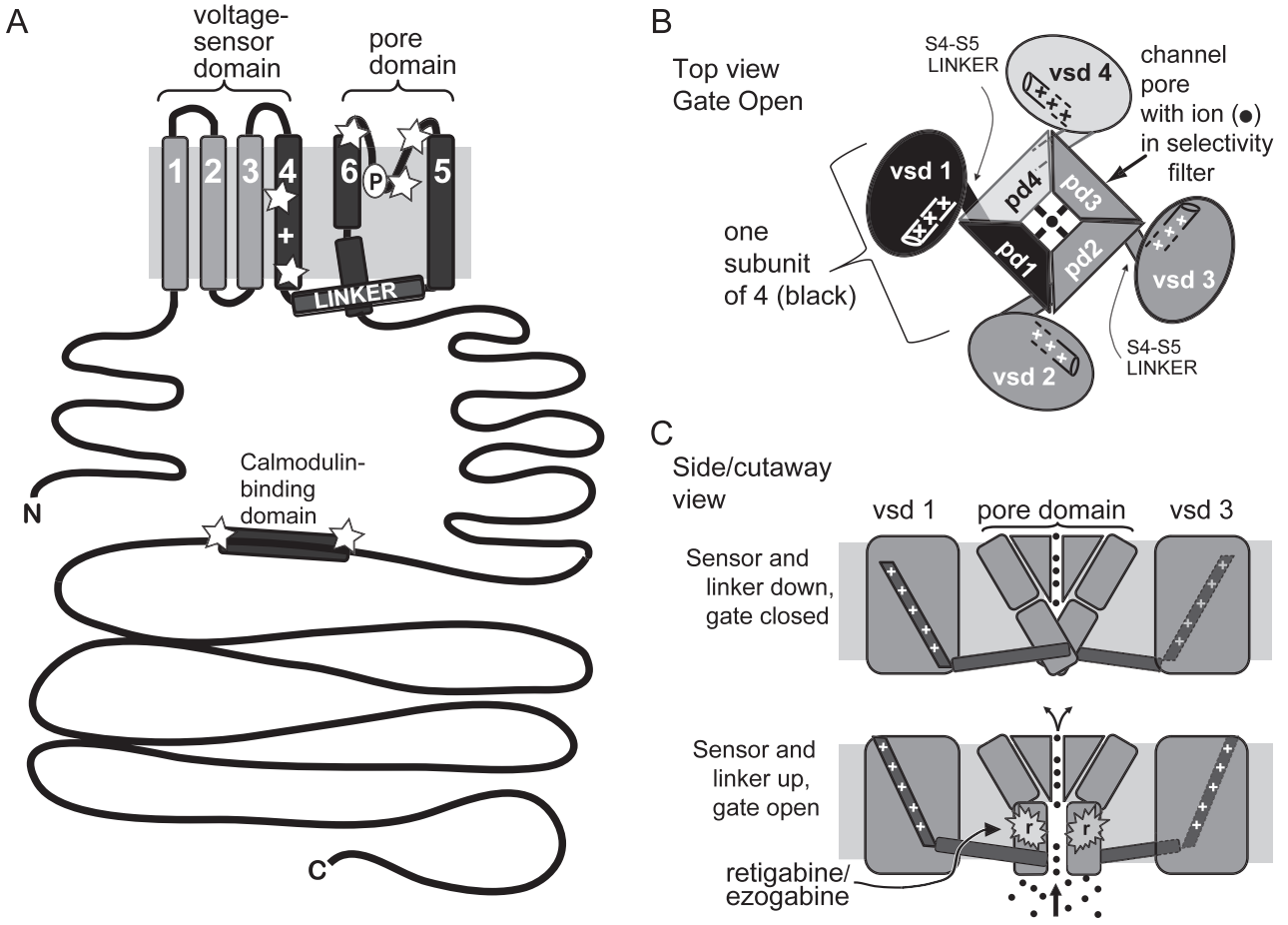

FIGURE 1. Mutations in KCNQ2 encephalopathy are clustered in critical gating and pore-forming functional domains. A. Cartoon showing the transmembrane folding of a KCNQ2 subunit, with intracellular amino (N) and carboxy (C) terminals, and six transmembrane helices that constitute voltage-sensor domain (S1-S4) and pore domain (S5-S6). Locations of KCNQ2 encephalopathy mutations are indicated by stars; the location of the dominant-negative mutation studied by Peters et al. (6) is an oval labeled P. Two additional mutations (not otherwise discussed here) adjoin a site that binds calmodulin (see [1]). B. Cartoon showing the tetrameric arrangement of subunits around the ion pore, viewed as from the cell exterior. The pore domains of the four subunits (labeled pd1-4) are central and interdigitated while the voltage sensor domains (vsd1-4) are peripheral. C. Cutaway side view of the channel illustrating how the coupled movement of the S4 helices, gating linkers, and pore gates bring about voltage-dependent closing and opening of the channel. Also shown is the location on the pore domain where retigabine/ezogabine (r) binds, stabilizing the open channel state (see [2]).

"electromechanical coupling" (4). The exact mechanism in each channel subunit is intricately engineered but simple in principle. One component, the S4 transmembrane helix, possesses positively charged arginine residues that pull the helix in response to membrane potential changes (see Figure 1). The pull is outward for depolarization and inward at rest. A second channel component, the S4-5 linker, is like a chain or lever connecting the voltage sensor to the actual gate. The gate is formed by part of the S6 transmembrane helix. Opening of the gate must also be coupled to an intact path for ions across the approximately 70 Angstrom thickness of the membrane. This path, the true "channel" or pore, is formed by parts of S5 and S6, and the peptide loop that connects them. Thus, a short stretch of amino acids from S4 through S6 is packed with key, coupled functions: voltage-sensing, gating, and ion conduction. Notably, six of the eight KCNQ2 encephalopathy patients show missense mutations in this functionally critical, precisely constructed region. By contrast, the heterozygous mutations in BFNS include complete deletion of the gene, and missense and nonsense mutations that are spread more widely throughout the subunit's sequence.

Heterozygous mutations that affect the coupling of voltage sensing to gating, or gating to conduction, can be more damaging than gene deletions if the mutant subunits can assemble with and poison the activity of normal subunits derived from the remaining wild-type gene. Each KCNQ channel is a tetramer, with each subunit contributing one of four walls of the transmembrane pore and its narrowest part, the potassium ion-selective filter (Figure 1B). Importantly, all four gates must move from closed to open, at the same time, for ions to flow. Because of this arrangement, one subunit bearing an electromechanically uncoupling or pore-blocking mutation

can render a tetramer formed with three wild-type partners completely nonconducting. Such coassembly and poisoning of wild-type subunits is termed a dominant-negative effect. If complete, it leads to a mathematically calculable eightfold greater reduction in functional channels compared with the case of heterozygous gene deletion (5). Such a strong reduction could explain the more severe phenotype of these newly described mutations.

Remarkably, a transgenic mouse model has already shown that a critically placed *KCNQ2* mutation can result in a severe, persistent epileptic encephalopathy-like phenotype (6). Peters et al. (2005) rationally designed a *KCNQ2* mutation predicted to block the channel pore and cause dominant-negative suppression of channel tetramers. The mutation replaced a single, very compact amino acid with a slightly bulkier one, precisely at the narrowest portion of the transmembrane ion path. Peters et al. expressed their "designer" mutant channel in cultured cells and found it was nonconducting. As hoped, the mutant also assembled with and suppressed the conduction of coexpressed wild-type subunits. They next generated transgenic mice bearing the mutant *KCNQ2* gene under control of an antibiotic-sensitive promoter. That arrangement allowed neuronal KCNQ channel function to be suppressed or allowed by withholding or administering the antibiotic. Without the antibiotic, hippocampal neurons showed a reduction in KCNQ-associated potassium currents and resultant increased cellular excitability. Impressively, mice expressing the mutant channel exhibited hippocampal heterotopias, early onset of persistent epilepsy, and marked disturbances in cognitive function during adulthood. Notably, the residue experimentally mutated by Peters et al. is very near three of the mutations uncovered by Weckhuysen et al. in their human patients (Figure 1A). It will be important to learn, in follow-up studies, whether these new human *KCNQ2* mutations cause epileptic encephalopathy through mechanisms analogous to those seen in the Peters et al. "designer" mouse.

Yet another potential pathogenic uncoupling that may be involved in these cases is the spatial separation of mutant KCNQ2-containing channels from their normal partner, the voltage-gated sodium (Na_V) channel. KCNQ2, KCNQ3, and Na_V channels are bound at axon initial segments and nodes of Ranvier to a common submembranous scaffold protein, ankyrin-G (7). Heterologous expression studies indicate that some BFNS mutations cause KCNQ2 and KCNQ3 to lose their ability to join Na_V channels clustered in the axonal membrane (8). Such loss of functional KCNQ channels adjoining Na_V channels in the axonal membrane has been shown to result in increased and pathologically sustained firing (9, 10). Of course, the two potential mechanisms, dominant-negative "poisoning" and mislocalization, could make additive contributions to the phenotype.

Importantly, Peters et al. found that preventing expression of their pore-blocking KCNQ2 mutant temporarily during early development was sufficient to prevent the anatomical disorganization and more severe behavioral effects otherwise seen. Ezogabine/retigabine can increase currents through neuronal KCNQ channels several-fold, potentially allowing even a small number of residual wild-type channel tetramers to act with far greater potency (2). Thus, elucidating the mechanisms of these novel human *KCNQ2* mutations has implications for diagnostic screening and therapy. For the moment, *KCNQ2* encephalopathy cannot be clinically differentiated from EME or Ohtahara syndrome at initial presentation. Therefore, it appears appropriate to include *KCNQ2* mutation screening early in the evaluation of these patients. Neonatal EEG is a potential biomarker for this condition if discriminating features within the burst suppression pattern can be identified.

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